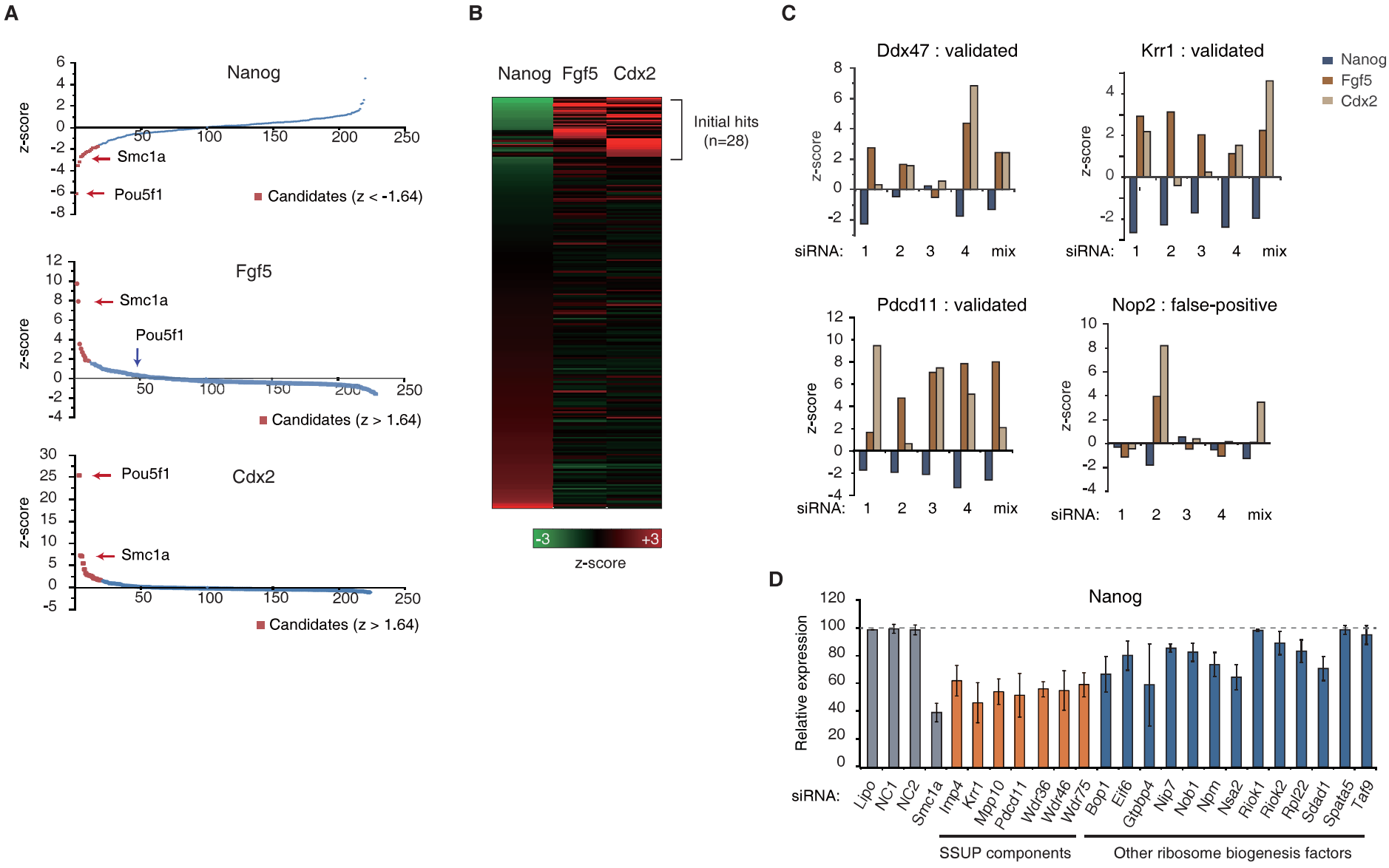
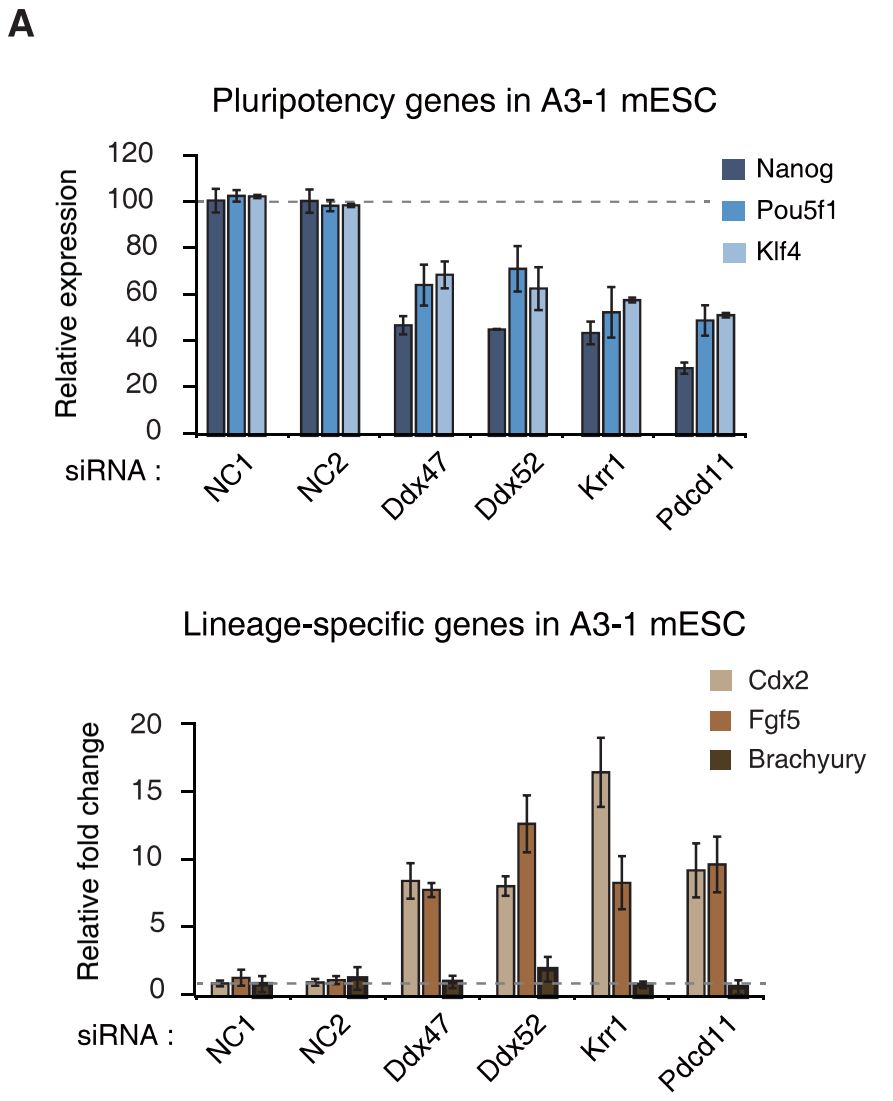
Supplemental Information

Supplemental information includes eleven figures, nine tables, supplemental experimental procedures, and supplemental references.


A
Nanog
z-score
Smc1a
Pou5f1
Candidates (z < -1.64)
Fgf5
Smc1a
Pou5f1
Candidates (z > 1.64)
Pou5f1
Cdx2
Smc1a
Candidates (z > 1.64)
B
Nanog Fgf5 Cdx2
Initial hits (n=28)
-3
+3
z-score
C
Ddx47 : validated
Krr1 : validated
Nanog
Fgf5
Cdx2
siRNA: 1 2 3 4 mix
Pdcd11 : validated
Nop2 : false-positive
D
Nanog
Relative expression
siRNA: Lipo NC1 NC2 Smc1a Imp4 Krr1 Mpp10 Pdcd11 Wdr36 Wdr46 Wdr75 Bop1 Eif6 Gtpbp4 Nip7 Nob1 Npm Nsa2 Riok1 Riok2 Rpl22 Sdad1 Spata5 Taf9
SSUP components
Other ribosome biogenesis factors


A
Pluripotency genes in A3-1 mESC
Relative expression
120
100
80
60
40
20
0
Nanog
Pou5f1
Klf4
siRNA :
NC1
NC2
Ddx47
Ddx52
Krr1
Pdcd11
Lineage-specific genes in A3-1 mESC
Relative fold change
20
15
10
5
0
Cdx2
Fgf5
Brachyury
siRNA :
NC1
NC2
Ddx47
Ddx52
Krr1
Pdcd11
B
siNC
siDdx47
siDdx52
siSmc1a
siKrr1
siPdcd11
C
Krr1/Ssea1/DAPI
Esrrb/Ssea1/DAPI
Tfcp2l1/Ssea1/DAPI
siNC
siKrr1

A

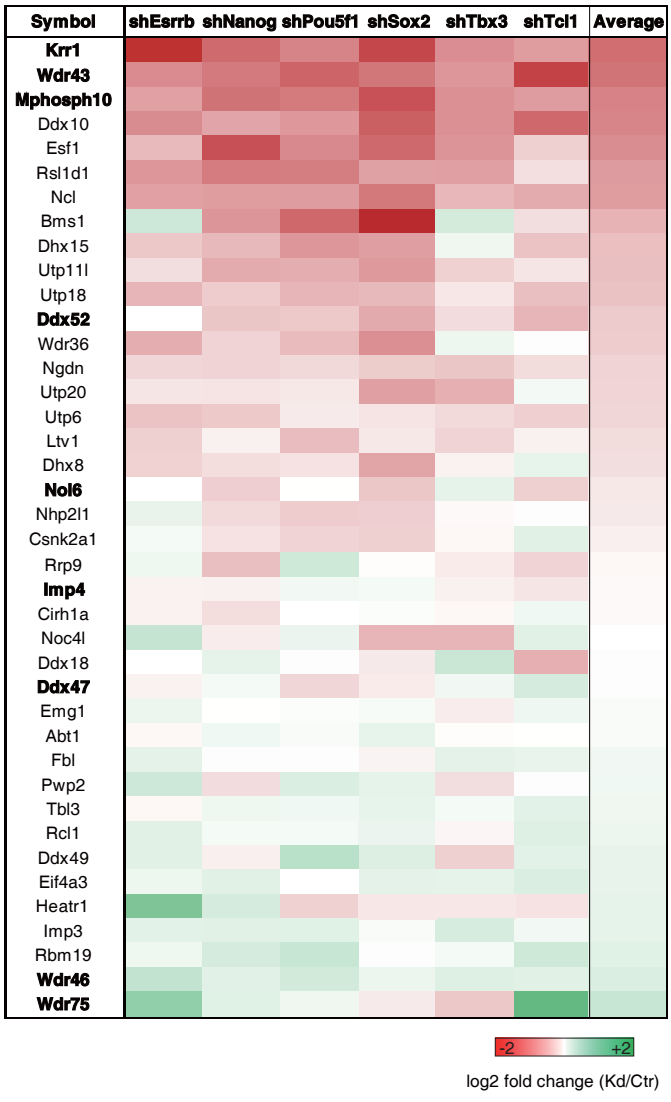
Comparison between DEGs from core TF knockdown and those from Krr1 knockdown

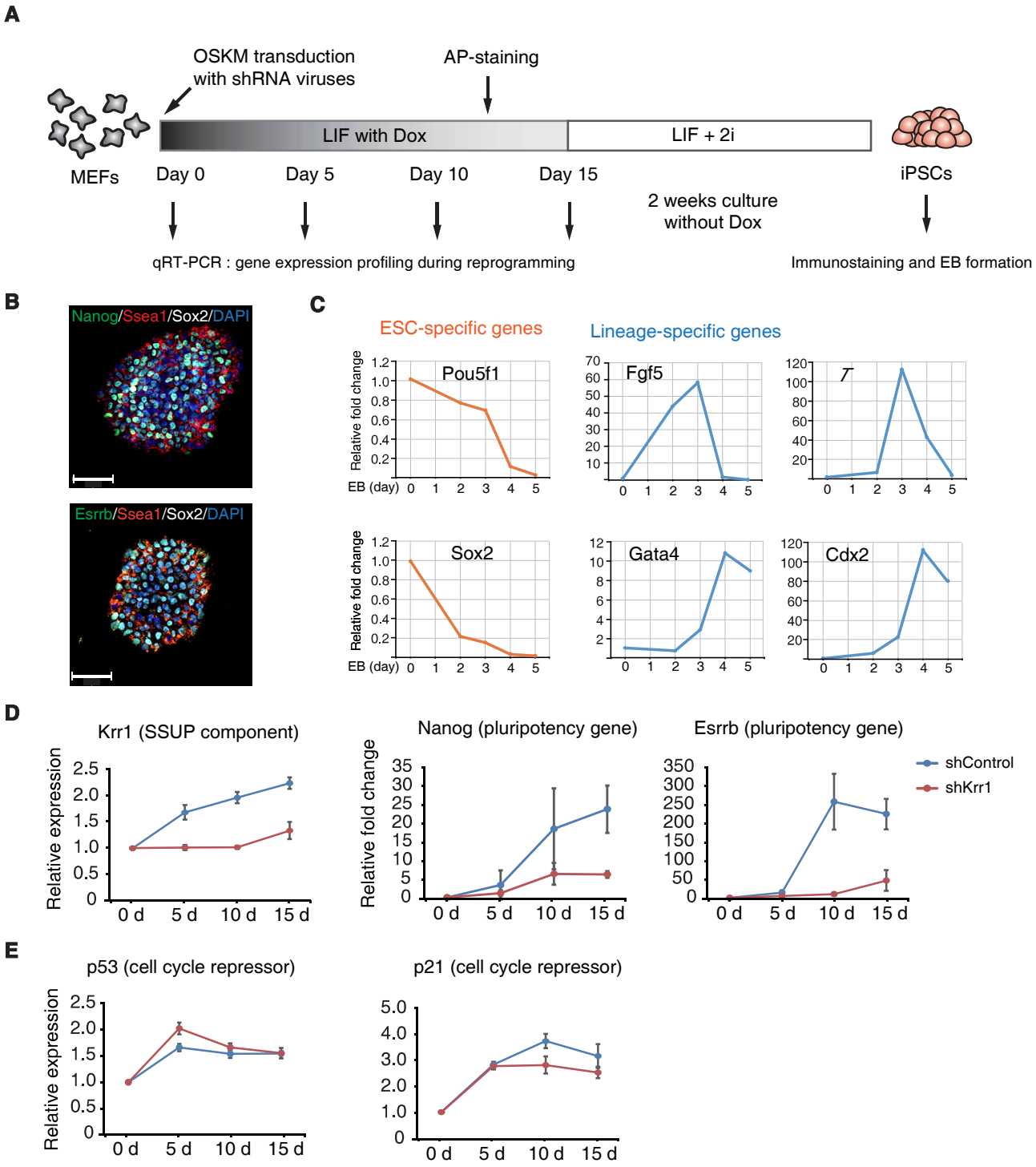
RNAi set	Overlap with DEGs from siKrr1	Odd ratio
Esrrb kd	p = 4.094e-62	7.143
Nanog kd	p = 5.521e-47	4.573
Pou5f1 kd	p = 2.678e-55	5.846
Sox2 kd	p = 1.740e-54	5.187
Tbx3 kd	p = 5.829e-38	5.492
Tcl1 kd	p = 1.483e-48	8.125

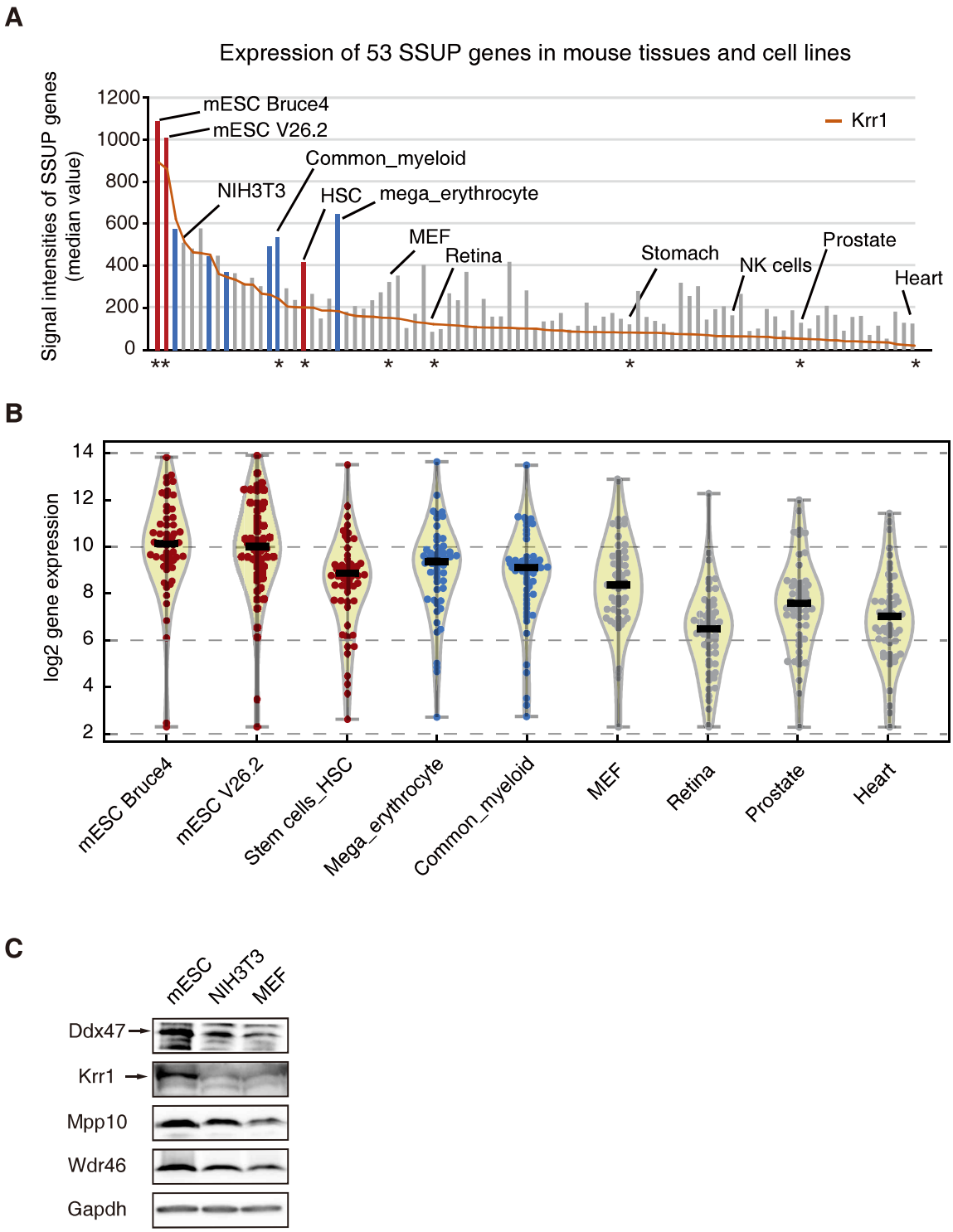
One-tailed Fisher's exact test

B

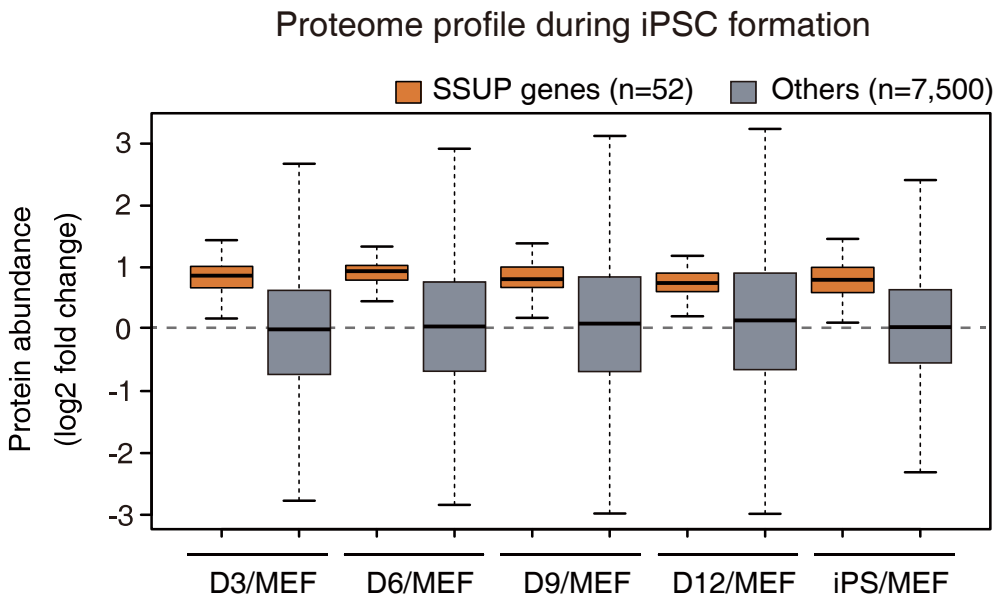
Changes of the mRNA levels of SSUP genes following core TF knockdown

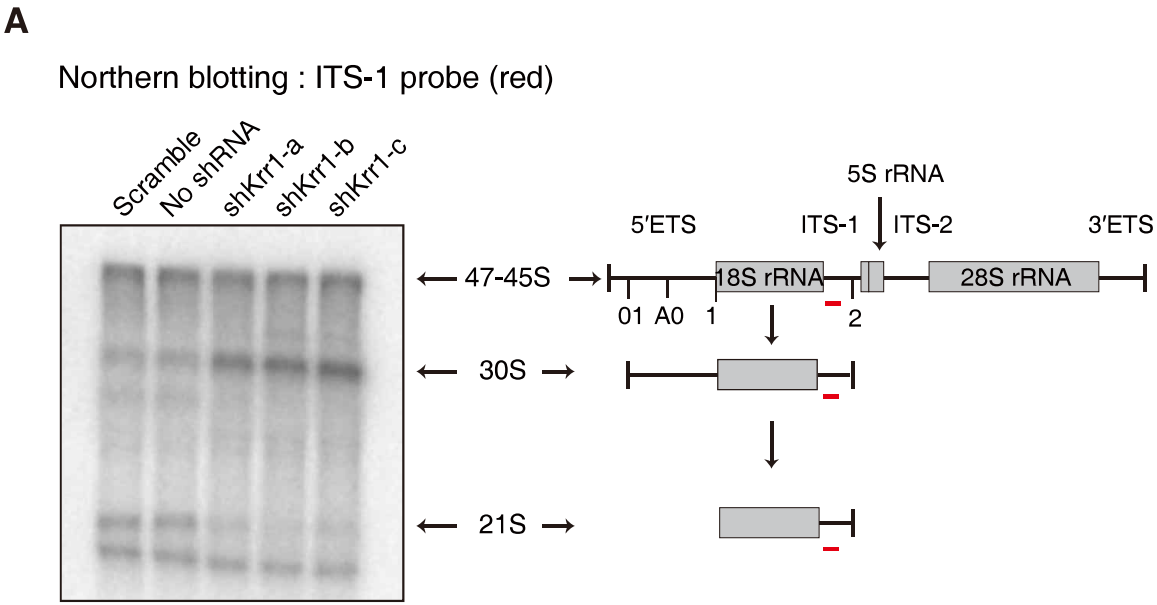


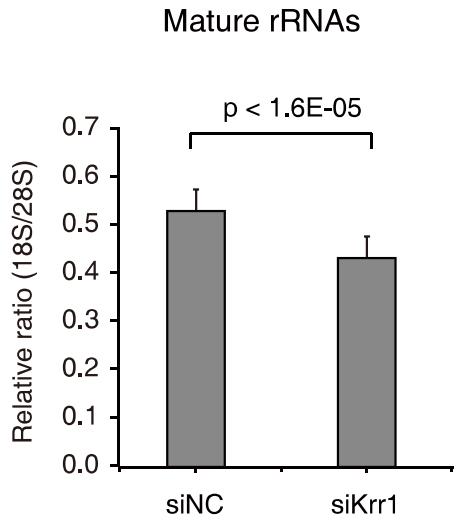

A
OSKM transduction
with shRNA viruses
AP-staining
LIF with Dox
LIF + 2i
MEFs
Day 0
Day 5
Day 10
Day 15
2 weeks culture
without Dox
iPSCs
qRT-PCR : gene expression profiling during reprogramming
Immunostaining and EB formation
B
Nanog/Ssea1/Sox2/DAPI
Esrrb/Ssea1/Sox2/DAPI
C
ESC-specific genes
Lineage-specific genes
Pou5f1
Fgf5
T
Sox2
Gata4
Cdx2
Relative fold change
EB (day)
D
Krr1 (SSUP component)
Nanog (pluripotency gene)
Esrrb (pluripotency gene)
Relative expression
Relative fold change
shControl
shKrr1
0 d
5 d
10 d
15 d
E
p53 (cell cycle repressor)
p21 (cell cycle repressor)
Relative expression

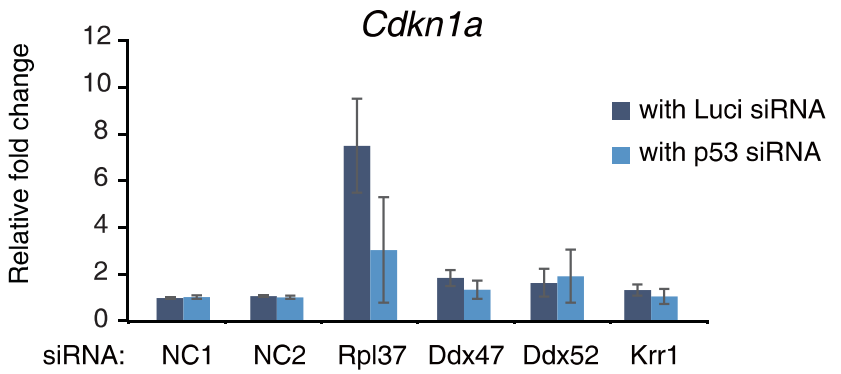

A
Expression of 53 SSUP genes in mouse tissues and cell lines
Signal intensities of SSUP genes (median value)
1200
1000
800
600
400
200
0
mESC Bruce4
mESC V26.2
Common_myeloid
NIH3T3
HSC
mega_erythrocyte
MEF
Retina
Stomach
NK cells
Prostate
Heart
Krr1
B
log2 gene expression
14
12
10
8
6
4
2
mESC Bruce4
mESC V26.2
Stem cells_HSC
Mega_erythrocyte
Common_myeloid
MEF
Retina
Prostate
Heart
C
mESC
NIH3T3
MEF
Ddx47
Krr1
Mpp10
Wdr46
Gapdh

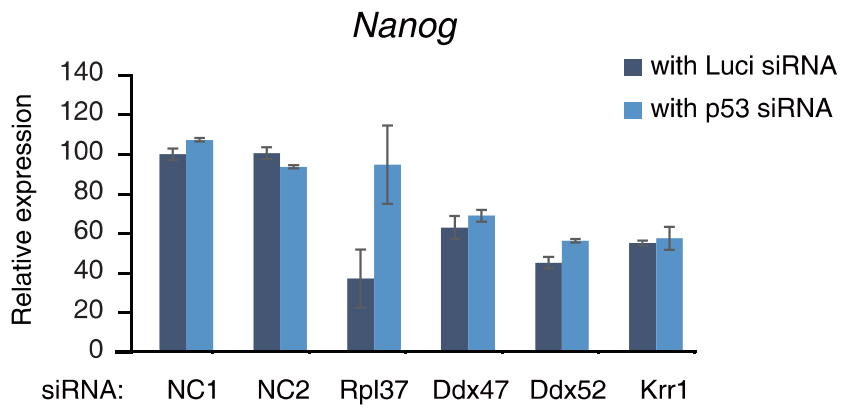
267112_Fig.S6_You



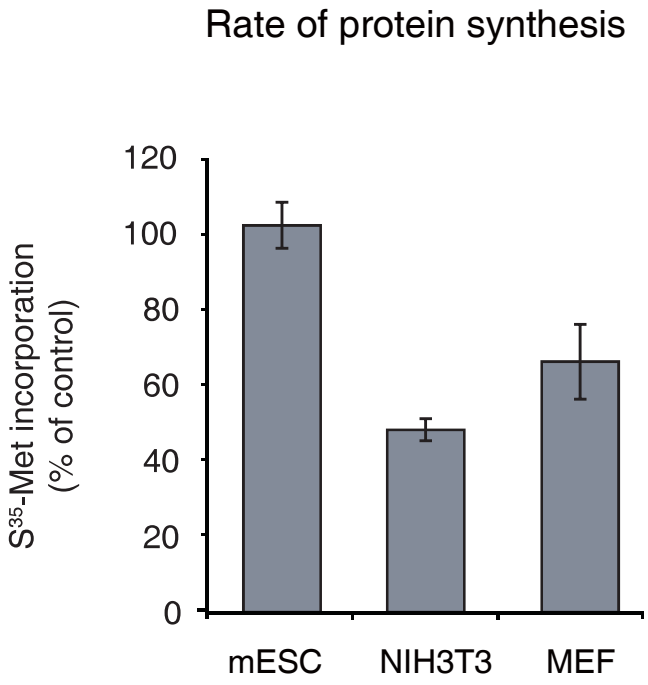

A
Northern blotting : ITS-1 probe (red)
Scramble
No shRNA
shKrr1-a
shKrr1-b
shKrr1-c
47-45S
30S
21S
5′ETS
18S rRNA
5S rRNA
ITS-1
ITS-2
28S rRNA
3′ETS
01
A0
1
2

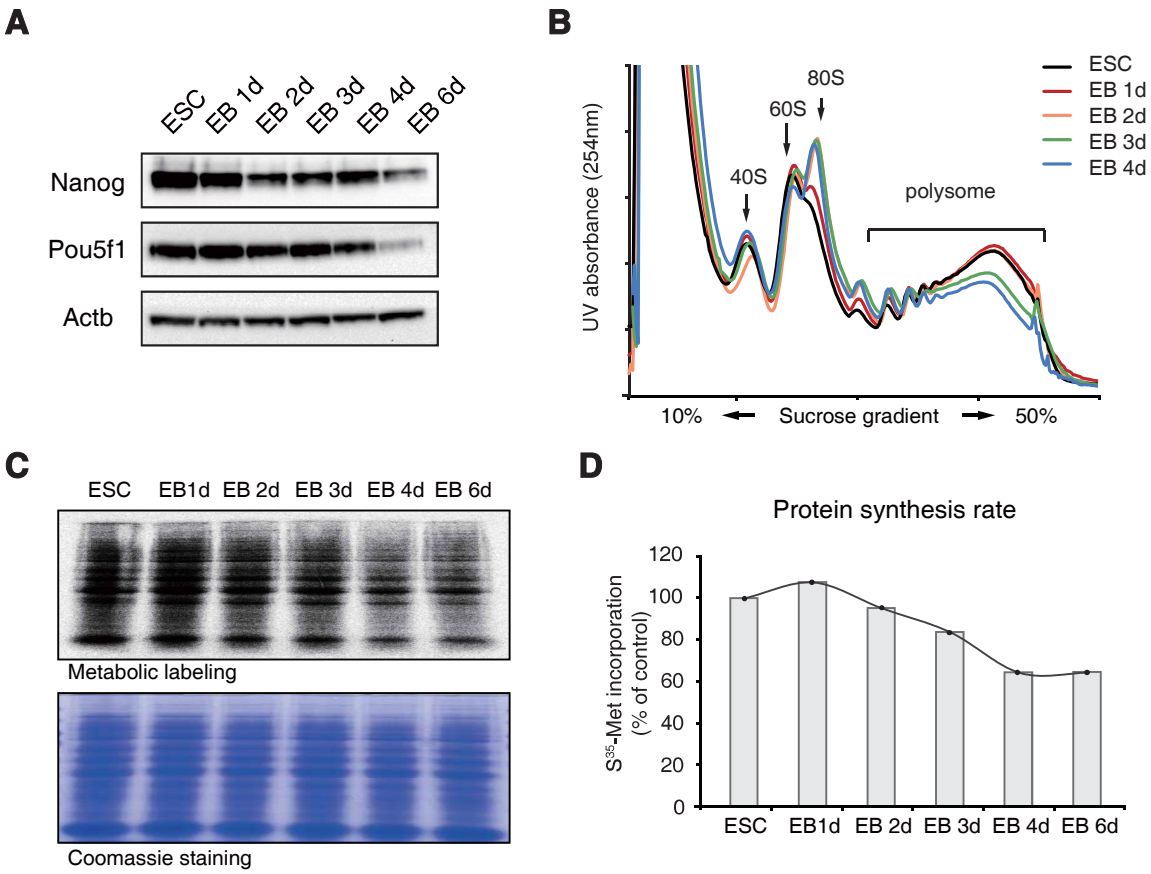

B
Mature rRNAs
p < 1.6E-05
Relative ratio (18S/28S)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
siNC
siKrr1

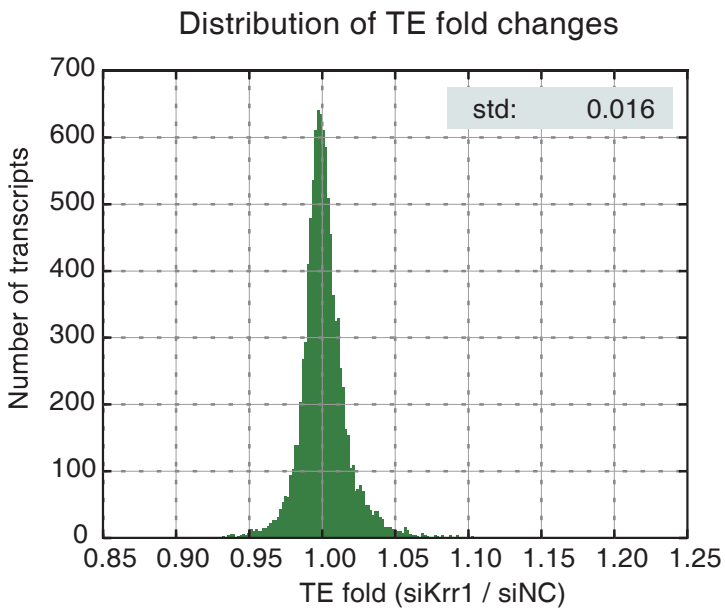

Cdkn1a
Relative fold change
12
10
8
6
4
2
0
with Luci siRNA
with p53 siRNA
siRNA:
NC1
NC2
Rpl37
Ddx47
Ddx52
Krr1


Nanog
Relative expression
140
120
100
80
60
40
20
0
with Luci siRNA
with p53 siRNA
siRNA:
NC1
NC2
Rpl37
Ddx47
Ddx52
Krr1

267112_Fig.S9_You




A
ESC
EB 1d
EB 2d
EB 3d
EB 4d
EB 6d
Nanog
Pou5f1
Actb
B
ESC
EB 1d
EB 2d
EB 3d
EB 4d
80S
60S
40S
polysome
UV absorbance (254nm)
10%
Sucrose gradient
50%
C
ESC
EB1d
EB 2d
EB 3d
EB 4d
EB 6d
Metabolic labeling
Coomassie staining
D
Protein synthesis rate
S35-Met incorporation (% of control)
120
100
80
60
40
20
0
ESC
EB1d
EB 2d
EB 3d
EB 4d
EB 6d


Distribution of TE fold changes
std: 0.016
Number of transcripts
700
600
500
400
300
200
100
0
0.85 0.90 0.95 1.00 1.05 1.10 1.15 1.20 1.25
TE fold (siKrr1 / siNC)

Supplemental Figure Legends

Supplemental Fig. S1. RNAi screening and validation of Initial hits, related to Fig. 1 (*A*) The average ranked z-score of each marker from the initial RNAi screen is plotted. The red circles indicate the initial hits. (*B*) Among 247 genes, 28 RBPs are selected as regulators of ESC status from initial screen. (*C*) Representative data from the secondary screen using four individual siRNAs. If more than 2 out of 4 siRNAs induce differentiation, the gene was considered as a positive hit. All the mRNA levels were normalized against *Actb* mRNA or others (when indicated). (*D*) qRT-PCR shows the relative *Nanog* expression levels after RNAi of ribosome biogenesis factors. The orange and blue bars denote SSUP and other ribosome biogenesis genes, respectively. All the error bars represent the mean ± SD from triplicates or duplicate experiments (when indicated).

Supplemental Fig. S2. SSUP genes are involved in regulation of ESC identity, related to Fig. 2 (*A*) Altered expression of ESC markers upon RNAi of SSUP genes in A3-1 mESCs. (*B*) Morphological changes upon knockdown of SSUP genes. RNAi of Smc1a was used as a control. Bars, 100 μm. (*C*) Immunostaining of Krr1 knockdown R1 mESCs for endogenous Krr1, Esrrb, and Tfcp2l1 (green), Ssea1 (red), and nuclei (blue). Scale bars, 50um.

Supplemental Fig. S3. Krr1 depletion impairs the expressions of pluripotency gene network, related to Fig. 2 (*A*) Overlaps between the DEGs from core TF knockdown and the DEGs from Krr1 knockdown. (*B*) Expression of SSUP components such as Krr1, Wdr43, and Mphosph10, upon knockdown of core ESC regulators (Ivanova et al. 2006). Bold symbols denote the SSUP genes that are shown to influence ESC maintenance in this study.

Supplemental Fig. S4. Experimental process of iPSC reprogramming by ectopic expression of defined factors, related to Fig. 2 (*A*) Schematic diagram of reprogramming procedure. (*B*) Immunostaining of derived iPSC clones for pluripotency markers. Nanog and Esrrb (green), Ssea1 (red), Sox2 (white), and nuclei (blue). Scale bars, 50um. (*C*) qRT-PCR shows the relative expression of pluripotency (Pou5f1, Sox2), ectoderm (Fgf5), mesoderm (*T*/brachyury), endoderm (Gata4), and extra-embryonic (Cdx2) markers during EB differentiation of derived iPSC clones. (*D* and *E*) qRT-PCR shows the expression change of several marker genes during reprogramming process of MEFs with shRNAs for negative control (shControl) or Krr1 (shKrr1). All the error bars represent the mean ± SD of triplicates.

Supplemental Fig. S5. SSUP is Preferentially Expressed in Pluripotent Stem Cells, related to Fig. 3 (*A*) The median expression values of 53 SSUP components in 89 mouse tissues and cell types are shown. Re-analysis of the microarray data from BioGPS, web-based database (Lattin et al. 2008; Wu et al. 2009). The red and blue bars denote stem cells and progenitor cells, respectively. The orange line indicates expression profile of Krr1. (*B*) Violin plot shows that expression pattern of 53 SSUP genes in representative tissues, which are indicated by an asterisk (*) in (*A*). Dots indicate the expression level of each SSUP components. (*C*) Western blotting validates the expression of SSUP genes in R1 mESC, NIH3T3, and MEF.

Supplemental Fig. S6. SSUP is upregulated during iPSC reprogramming, related to Fig. 3 Proteome profiles during iPSC reprogramming indicate that the SSUP components are up-regulated in early phase and the enhanced levels are maintained during the reprogramming

process (Hansson et al. 2012). D denotes days after reprogramming, MEF denotes mouse embryonic fibroblast.

Supplemental Fig. S7. Krr1 is required for pre-18S rRNA processing in mESCs, related to Fig. 4 (*A*) Analysis of pre-rRNA processing by Northern blotting. Lentiviruses expressing three different shRNA for Krr1 are transduced in R1 cells, respectively. A schematic representation of pre-rRNA processing intermediates is shown on the right side. The position of ITS-1 probe is indicated as a red bar. (*B*) Knockdown of Krr1 results in the reduction of the ratio of 28S rRNA versus 18S rRNA as measured by microfluidics-based electrophoresis.

Supplemental Fig. S8. The p53-dependent ribosomal stress pathway is not required for reduction of Nanog in SSUP knockdown cells. Simultaneous knockdown of p53 does not rescue the reduction of Nanog expression in SSUP-depleted cells. Expression level of genes was measured two days after siRNA transfection. Induction of Cdkn1a/p21 expression is a mark of p53 activation. Knockdown of Rpl37 is used as a control that triggers the p53-dependent ribosomal stress response in mESCs (Morgado-Palacin et al. 2012).

Supplemental Fig. S9. Mouse ESC has higher protein synthesis rate compared to NIH3T3 and MEF, related to Fig. 4 Protein synthesis rate in different cell lines were measured by S^{35}-methionine incorporation assay.

Supplemental Fig. S10. General translational capacity is decreased during exit from pluripotency, related to Fig. 4 (*A*) Western blotting determines the protein levels of Nanog

and Pou5f1 during EB differentiation. (*B*) Polysome profiles show the decreased polysome content during early phase of differentiation. (*C* and *D*) Metabolic labeling shows the change of protein synthesis rate during EB differentiation.

Supplemental Fig. S11. The distributions of fold change for translation efficiency in response to Krr1-knockdown, related to Fig. 4

Supplemental Tables

Supplemental Table S1. List of 247 RBPs for RNAi screen, related to experimental procedures

Supplemental Table S2. Results of RNAi screen (z-score values for each markers), related to Fig. 1

Supplemental Table S3. Initial 28 hits from the RNAi screen, related to Supplemental Fig. S1

Supplemental Table S4. List of genes excluded due to cell death, related to Supplemental Fig. S1

Supplemental Table S5. List of final 16 RBPs important for pluripotency maintenance, related to Fig. 1

Supplemental Table S6. Putative components of SSUP used in this study

Supplemental Table S7. Differentially expressed genes from RNA-seq, related to Fig. 2

Supplemental Table S8. Gene set enrichment analysis, related to Fig. 2

Supplemental Table S9. Oligonucleotides used in this study, related to experimental procedures

***Supplemental Table S1, S2, S6, and S7 are available as separate Excel files:**

Supplemental Table S3. Initial 28 hits from the RNAi screen, related to Supplemental Fig. S1

Symbol	Accession No.	Name	Notes (Related to pluripotency or lncRNA)
Cnot6*	NM_212484	CCR4-NOT transcription complex, subunit 6	
Cwc15	NM_023153	CWC15 homolog (S. cerevisiae)	
Ddx47	NM_026360	DEAD (Asp-Glu-Ala-Asp) box polypeptide 47	Pou5f1-interaction
Ddx52	NM_030096	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52	
Ddx56	NM_026538	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56	
Dkc1	NM_001030307	Dyskeratosis congenita 1, dyskerin homolog (human)	Dkc1-mutant iPSCs show telomere shortening and loss of self-renewal
Exosc8	NM_027148	Exosome component 8	
Exosc9	NM_019393	Exosome component 9	Necessary for the self-renewal of epidermal progenitor cells
Hnrnpk*	NM_025279	Heterogeneous nuclear ribonucleoprotein K	Regulates lncRNA-p21 or lncRNA *TUNA*
Hnrnpu*	NM_016805	Heterogeneous nuclear ribonucleoprotein U	Interact with lncRNA Firre, Pou5f1-interaction
Krr1	NM_178610	KRR1, small subunit (SSU) processome component, homolog (yeast)	
Nifk*	NM_026472	Mki67 (FHA domain) interacting nucleolar phosphoprotein	Suggest the functional importance of nucleolus in pluripotency
Nol6	NM_139236	Nucleolar protein family 6 (RNA-associated)	
Nop2	NM_138747	NOP2 nucleolar protein homolog (yeast)	Interacts with lncRNA-hPVT1
Pabpn1	NM_019402	Poly(A) binding protein, nuclear 1	Promotes lncRNA turnover
Pdcd11	NM_011053	Programmed cell death 11	

Poldip3	NM_178627	Polymerase (DNA-directed), delta interacting protein 3	
Ptbp1*	NM_001077363	Polypyrimidine tract binding protein 1	Regulates lncRNA *TUNA*
Rbm19	NM_028762	RNA binding motif protein 19	
Rbm28	NM_133925	RNA binding motif protein 28	
Rbm42	NM_133693	RNA binding motif protein 42	
Rrp7a	NM_029101	Ribosomal RNA processing 7 homolog A (S. cerevisiae)	
Rrp9	NM_145620	RRP9, small subunit (SSU) processome component, homolog (yeast)	
Srbd1	NM_030133	S1 RNA binding domain 1	
Srrm1	NM_016799	Serine/arginine repetitive matrix 1	
Srsf7	NM_146083	Serine/arginine-rich splicing factor 7	
Tardbp*	NM_001003898	TAR DNA binding protein	
Zc3h14	NM_029334	Zinc finger CCCH type containing 14	

* = identified genes for pluripotency in previous RNAi screens

Supplemental Table S4. List of genes excluded due to cell death, related to Supplemental Fig. S1

Label	Accession No.	Symbol	Notes (Related to pluripotency or lncRNA)
146M	NM_025323	*0610009d07rik*	
109M	NM_009702	*Aqr*	
174M	NM_025820	*Crnkl1*	
190M	NM_007916	*Ddx19a*	
10M	NM_028041	*Ddx54*	
12M	NM_144831	*Dhx8*	

14M	NM_138669	*Eif4a3*	
54M	NM_025513	*Exosc3*	
125M	NM_010760	*Magoh*	
23M	NM_010880	*Ncl**	Regulates lncRNA *TUNA*, role in p53 activation
45M	NM_008774	*Pabpc1*	Interacts with KSHV viral lncRNA PAN
194M	NM_021568	*Pcbp3*	
120M	NM_025443	*Pno1*	
98M	NM_026329	*Polr2g*	
205M	NM_027328	*Prpf31*	
95M	NM_138659	*Prpf8*	
124M	NM_028364	*Puf60*	Interacts with lncRNA and regulates translation of a subset of genes
241M	NM_025776	*Rbm22*	
130M	NM_133242	*Rbm39*	
72M	NM_020600	*Rps14*	
71M	NM_009091	*Rps15*	
136M	NM_026147	*Rps20*	
33M	NM_026175	*Sf3a1**	
77M	NM_009224	*Snrnp70*	
118M	NM_021336	*Snrpa1*	
182M	NM_011432	*Snrpc*	
114M	NM_009227	*Snrpe*	
35M	NM_027246	*Snrpf*	
195M	NM_178880	*Son**	Regulates proper splicing of ESC factors in hESCs
117M	NM_173374	*Srsf1*	Interact with MALAT1 and organize speckle assembly
46M	NM_011358	*Srsf2*	
36M	NM_013663	*Srsf3**	
131M	NM_009297	*Supt6h*	

116M	NM_011568	*Thoc4*	
230M	NM_024187	*U2af1*	
67M	NM_133671	*U2af2*	
123M	NM_134014	*Xpo1*	

* = identified genes for pluripotency in previous studies

Supplemental Table S5. List of final 16 RBPs important for pluripotency maintenance, related to Fig. 1

Symbol	Aliases	Name	Known Functions, notes	Subcellular Location
Cwc15	mED1, Cwf15, Ed1	CWC15 homolog (S. cerevisiae)	splicing	Nu, Mt
Ddx47	E4-DBP, RRP3	DEAD (Asp-Glu-Ala-Asp) box polypeptide 47	rRNA processing (SSUP), Pou5f1-interaction	No
Ddx52	HUSSY19, ROK1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52	rRNA processing (SSUP)	Nu, No
Ddx56	DDX21, DDX26, Noh61	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56	rRNA processing	Nu, No*
Hnrnpu	Hnrpu, SAFA, Sp120	Heterogeneous nuclear ribonucleoprotein U	splicing, lncRNA regulation, Pou5f1-interaction	Nu
Hnrnpk	CSBP, KBBP, NOVA, Hnrpk	Heterogeneous nuclear ribonucleoprotein K	splicing, role in p53 response, lncRNA regulation	Nu
Krr1	HRB2, RIP-1	KRR1, small subunit (SSU) processome component	rRNA processing (SSUP)	Nu, No
Nifk	Mki67ip, Nopp34	Mki67 (FHA domain) interacting nucleolar phosphoprotein	rRNA processing, role in mitosis	No
Nol6	NRAP, UTP22	Nucleolar protein family 6 (RNA-associated)	rRNA processing (SSUP)	Nu, No, Mt
Pdcd11	ALG4, NFBP, RRP5, Pdcd7	Programmed cell death 11	rRNA processing (SSUP)	No
Ptbp1	HNRPI, PTB-1, PTB2, PTB3, PTB4, Ptb, pPTB	Polypyrimidine tract binding protein 1	splicing, lncRNA regulation	Nu
Rbm28	ANES, 2810480G15Rik	RNA binding motif protein 28	rRNA processing	No
Rbm42	1700003D06Rik	RNA binding motif protein 42	splicing, 3′UTR binding	Nu, Cy
Rrp7a	Kheg1	Ribosomal RNA	rRNA processing	Nu, No

		processing 7 homolog A (S. cerevisiae)	(SSUP)	
Srsf7	Sfrs7, 9G8, NX-96, AAG3	Serine/arginine-rich splicing factor 7	splicing, mRNA export	Nu
Tardbp	TDP-43,Tdp43, ALS10	TAR DNA binding protein	splicing, 3′UTR binding	Nu

Nu = Nucleus, No = Nucleolus, Cy = Cytoplasm, Mt = Mitochondria, *= by similarity

Supplemental Table S8. Gene set enrichment analysis, related to Fig. 2

Category	**GO Term**	**P-value**
GO_Functional Annotation Clustering with Downregulated Genes in RNAseq	GO:0043228~non-membrane-bounded organelle	1.99E-06
	GO:0048863~stem cell differentiation	5.06E-04
	GO:0065003~macromolecular complex assembly	1.64E-05
	GO:0006412~translation	6.39E-04
	GO:0001568~blood vessel development	9.39E-04
	GO:0003700~transcription factor activity	0.00127
	GO:0001829~trophectodermal cell differentiation	0.00464
	GO:0009792~embryonic development ending in birth or egg hatching	0.00557
GO_Functional Annotation Clustering with Upregulated Genes in RNAseq	GO:0007155~cell adhesion	1.29E-13
	GO:0070161~anchoring junction	4.71E-10
	GO:0001944~vasculature development	7.70E-05
	GO:0043067~regulation of programmed cell death	1.72E-05
	GO:0035239~tube morphogenesis	6.44E-05

	GO:0004672~protein kinase activity	3.48E-05
	GO:0007010~cytoskeleton organization	3.99E-04
	GO:0016477~cell migration	3.83E-04

Supplemental Table S9. Oligonucleotides used in this study, related to experimental procedures

PCR primers for qRT-PCR		
Name	**Note**	**Sequence**
Nanog	forward	TTGCTTACAAGGGTCTGCTACT
	reverse	ACTGGTAGAAGAATCAGGGCT
Fgf5	forward	TGTGTCTCAGGGGATTGTAGG
	reverse	AGCTGTTTTCTTGGAATCTCTCC
Cdx2	forward	CAAGGACGTGAGCATGTATCC
	reverse	GTAACCACCGTAGTCCGGGTA
Klf4	forward	AGGAACTCTCTCACATGAAGCG
	reverse	GGTCGTTGAACTCCTCGGTC
Tbx3	forward	ACTCGGGGTCGGAACTGAA
	reverse	GGAGGGGGCGATTTTGTTTTT
Pou5f1	forward	CACCATCTGTCGCTTCGAGG
	reverse	AGGGTCTCCGATTTGCATATCT
Brachyury	forward	CTCGGATTCACATCGTGAGAG
	reverse	AAGGCTTTAGCAAATGGGTTGTA
Gata6	forward	TTGCTCCGGTAACAGCAGTG
	reverse	GTGGTCGCTTGTGTAGAAGGA
Gapdh	forward	AGGTCGGTGTGAACGGATTTG
	reverse	TGTAGACCATGTAGTTGAGGTCA

Actb	forward	GGCTGTATTCCCCTCCATCG
	reverse	CCAGTTGGTAACAATGCCATGT
45S pre-rRNA 5′ETS	forward	GGTTGAGGGCCACCTTATTT
	reverse	GGAGGCATGTGGAAGAAAGA
45S pre-rRNA 3′ETS	forward	ACGACCGTGTGGTGGTTG
	reverse	CTCCCACACCCAAGGAGAG

Oligonucleotides for construction of shRNA vectors

Name	Note	Sequence
Krr1 shRNA-1	forward	CCGGGCTCTCTAGTGAGAAATAAAGCTCGAGCTTTATTTCTCACTAGAGAGCTTTTTG
	reverse	AATTCAAAAAGCTCTCTAGTGAGAAATAAAGCTCGAGCTTTATTTCTCACTAGAGAGC
Krr1 shRNA-2	forward	CCGGGCCAGAGAGTCAGATTGATAACTCGAGTTATCAATCTGACTCTCTGGCTTTTTG
	reverse	AATTCAAAAAGCCAGAGAGTCAGATTGATAACTCGAGTTATCAATCTGACTCTCTGGC
Ddx47 shRNA-1	forward	CCGGGGATTATATCCATCGAGTTGGCTCGAGCCAACTCGATGGATATAATCCTTTTTG
	reverse	AATTCAAAAAGGATTATATCCATCGAGTTGGCTCGAGCCAACTCGATGGATATAATCC
Ddx47 shRNA-2	forward	CCGGGCTCACGGAACGTGTGAATGACTCGAGTCATTCACACGTTCCGTGAGCTTTTTG
	reverse	AATTCAAAAAGCTCACGGAACGTGTGAATGACTCGAGTCATTCACACGTTCCGTGAGC
Ddx52 shRNA-1	forward	CCGGGGACTAGACTTCTTCGGAAACCTCGAGGTTTCCGAAGAAGTCTAGTCCTTTTTG
	reverse	AATTCAAAAAGGACTAGACTTCTTCGGAAACCTCGAGGTTTCCGAAGAAGTCTAGTCC
Ddx52 shRNA-2	forward	CCGGGGAGAGAAGAAGTTAACTTCACTCGAGTGAAGTTAACTTCTTCTCTCCTTTTTG
	reverse	AATTCAAAAAGGAGAGAAGAAGTTAACTTCACTCGAGTGAAGTTAACTTCTTCTCTCC
Pdcd11 shRNA-1	forward	CCGGACACCAAGTCCGAGAAATACACTCGAGTGTATTTCTCGGACTTGGTGTTTTTTG
	reverse	AATTCAAAAAACACCAAGTCCGAGAAATACACTCGAGTGT

		ATTTCTCGGACTTGGTGT
Pdcd11 shRNA-2	forward	CCGGGCAACCAAGACTCTGTATTGACTCGAGTCAATACAG AGTCTTGGTTGCTTTTTG
	reverse	AATTCAAAAAGCAACCAAGACTCTGTATTGACTCGAGTCA ATACAGAGTCTTGGTTGC

Supplemental Experimental Procedures

Cell Culture and Differentiation

R1 mouse embryonic stem cells were maintained on 0.1% gelatin-coated dishes with ESC media (DMEM containing 15% FBS (Gibco), 1X nonessential amino acids (Gibco), 100 uM 2-mercaptoethanol (Sigma), and 1000 U/ml LIF (Millipore)). For retinoic acid (RA) differentiation, ESC cultured with ESC media without LIF, and containing 1 uM RA. For embryoid body (EB) differentiation culture, ESCs were suspension cultured on petri dishes with ESC media without LIF and split every 2 days. For inhibition of general translation, ESCs were cultured 3 days in ESC culture media containing 50 uM 4EGI-1.

RNAi Library Construction and RNAi Screen

Among 443 annotated RBPs in the uniprot database, we selected 247 RBPs according to its expression level in mESCs (See also Supplemental Table S1). We designed 4 different 21-mer RNA duplex for each of the 247 RBPs using block-it RNAi designer (Invitrogen). R1 cells were reverse transfected as follows: final 20 nM siRNA and one ul Lipofectamine 2000 were mixed with 100 ul Opti-MEM in each well of a 24-well plate. After 15 min incubation, 1.0

x 10^4 cells with 300 ul medium were seeded in each well. Luciferase siRNA, negative control siRNA (Bioneer), or without siRNA (Lipofectamine only) were used as negative controls. Smc1a siRNA was used as a positive and normalization control for plate batch variation. Cells were incubated in ESC medium for 4 days and harvested for RNA extraction. When we observed the apoptotic cell debris by light microscopy and the total amount of RNA is lower than 10 % of control set, we designated the siRNA as causing "severe" cell death and hence excluded them from the subsequent experiments even when the marker expression indicated differentiation (Table S4).

Quantitative Real-Time PCR

RNA was isolated the illustra RNAspin Kit (GE Healthcare), according to the manufacturer's instructions, and cDNA was produced with the Primescript RT Kit (Takara). Quantitative Real-time PCR reactions were set up in duplicate or triplicate with the Brilliant II SYBR Premix EX Taq Kit (Takara) and run on a StepOnePlus System (Applied Biosystems). Relative expression level of each gene was calculated using the comparative *Ct* method with *Actb* or *Gapdh* as a normalization control. Primer sequences for qPCR were taken from PrimerBank (Wang and Seed 2003) and are listed in Supplemental Table S9.

Construction of RNA-seq and Ribosome Profiling Library

RNA-seq and Ribosome profiling libraries were prepared as described previously (Cho et al. 2012), according to the protocol of ARTseq Ribosome Profiling Kit (Epicentre) with minor modifications. Briefly, R1 cells were transfected with three different siRNAs targeting controls and 3 different siRNAs targeting Krr1 for 48 hrs. Cell lysates were divided equally for RNA-seq library and ribosome profiling library. Lysates were digested by RNase I (Ambion) and

gel purified in the range of 30 nt for ribosome profiling or 40-60 nt for RNA-seq library. The purified RNAs were subjected to reverse-transcription, after ligated with 3′ and 5′ adapter. The cDNA was used as a template for PCR amplification using Phusion polymerase (NEB). Reactions were separated by acrylamide gel and purified again. Quantify and characterize the purified cDNA libraries using bioanalyzer (Agilent) and Kapa library quantification kit, respectively. The libraries were sequenced on Illumina HiSeq 2000 (multiplexing 50 base single-end). All adapters and primers were synthesized by IDT.

Classification of SSUP components

About 72 proteins were previously reported to be required for 18S rRNA processing and associated with U3 snoRNA and Mpp10 (Phipps et al. 2011). Among 72 putative SSUP components, 54 genes showed the defects of pre-rRNA processing upon RNAi transfection in human cells (Tafforeau et al. 2013). We used these 54 genes as components of SSUP in this study (Supplemental Table S6).

Bioinformatics Analysis

To analyze the mRNA profile during EB differentiation presented in Fig. 3B, we used the published data from previous RNA-seq study (Liu et al. 2011). For the proteome profile during reprogramming into iPSCs, we used the published proteome data (Hansson et al. 2012). These data were imported into the R program for further analysis shown in Fig. 3B and Supplemental Fig. S6~~5~~.

Expression levels of SSUP genes in various tissues are re-analyzed based on the preprocessed version of microarray data from GSE10246, provided by BioGPS (Lattin et al. 2008; Wu et al. 2009). Probe annotations are obtained from the platform GPL1261, and

probe sets suffixed by " _s_at" and "_x_at" are excluded to guarantee that the one gene per one probe. The median of probe intensities are assigned to each gene if a certain gene has multiple probes. Among 54 SSUP genes, Nom1 is excluded from this analysis because of no probe annotation is available. Summarized expression levels are then subjected to show SSUP gene expression profiles in various tissues (Fig. 3C and Supplemental Fig. S54).

Sequencing reads from RNA-seq firstly preprocessed to keep only high-quality reads. Preprocess carried out by applying trimming low-quality bases from 3′ end, adapter clipping, filtering out low-quality or artifacts, sequentially, using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Remained high-quality reads are then aligned to mouse genome, mm10, with STAR aligner (Dobin et al. 2013). We used Illumina iGenome as a reference annotation, and counted reads for each gene using HTSeq (Anders et al. 2015). Read counts are further normalized by upper quartile. RPF sequences are processed in the same manner, and translational efficiency is calculated as simply normalized RNA-seq read counts over normalized RPF read counts (Fig. 4E, 4F, and Supplemental Fig. S119).

For DEG analysis, lipofectamine-only, siGFP, negative control siRNA samples are considered as replicates for the control, and the rest as replicates for Krr1-depleted. The categorized read counts are then fed up to DESeq R package to obtain the list of differentially expressed genes (Anders and Huber 2010) (Fig. 2D). To examine how significant the overlap between our DEGs and the affected genes from depletion of pluripotency factors is (Ivanova et al. 2006), we compared the gene lists to generate 2x2 contingency table and then performed one-tailed Fisher's exact test (Supplemental Fig. S43A).

Gene Ontology analysis was performed with the web-based bioinformatics resources DAVID 6.7 (Huang da et al. 2009) using the functional annotation clustering for biological process, molecular function, and cellular component. We selected top 8 annotation cluster based on the enrichment score. Most significant GO term of each cluster are listed in

Supplemental Table S8.

S^{35}-Methionine Metabolic Labeling

R1 mESCs transfected with siRNA targeting control or RBPs for 48 hr, and incubated with media lacking methionine for 40 min. S^{35}-labeled methionine was added to the media and incubated for additional 30 min. Cells were harvested and the protein concentration were determined by BCA assay in triplicates. Equal amount of protein were separated by 10% SDS-PAGE and exposed to an imaging plate (Fujifilm). Radioisotope signals were read with the BAS-2500 system and the signal intensities were quantified using Multi Gauge (Fujifilm).

Reprogramming Assay and Alkaline Phosphatase Staining

To produce infectious viral particles, 293T cells cultured on 10 cm dishes were transfected with the TetO-FUW-OSKM, FUW-M2rtTA (Addgene plasmid 20321 and 20342) (Takahashi and Yamanaka 2006; Carey et al. 2009) or pLKO-shRNA vectors together with the packaging plasmids psPAX2 and pMD2.G (Addgene plasmid 10878, 12260 and 12259) using Fugene HD (Roche). Viral supernatants were harvested on three consecutive days starting 24 hrs after transfection, concentrated by centrifugation followed mixing with Lenti-X concentrator (Clontech). Carefully remove supernatant, viral particles were resuspended in Opti-MEM, and stored at -80˚C in single-use aliquots.

For reprogramming, MEFs were infected with lentiviruses containing TetO-FUW-OSKM (containing *Pou5f1, Sox2, Klf4*, and *c-Myc*) or FUW-M2rtTA in ESC medium containing LIF and doxycycline (Dox). For SSUP knockdown experiment, MEFs were re-infected with lentiviruses encoding shRNAs for Ddx47, Ddx52, Krr1, Pdcd11, and controls, 2 days later of OSKM transduction. Sequences of the shRNAs used are listed in Supplemental

Table S9. Fifteen days after Dox induction, mESCs were fixed 4% paraformaldehyde (PFA) and colonies were stained for alkaline phosphatase (AP) Activity. The number of AP positive colonies were counted by Image J. For immunostaining of iPSCs, reprogrammed cells were further cultured for 2 weeks without Dox treatment.

Western Blotting

Samples were resolved on 10% SDS-polyacrylamide gels or 4%–12% NuPAGE gels (Invitrogen) and transferred to Immobilon-P transfer membrane (Millipore) or Hybond C extra-western membrane (Amersham). Primary antibodies used in this study were goat anti-Sox2 (Santa Cruz), mouse anti-Gapdh (Santa Cruz), mouse anti-Mpp10 (Santa Cruz), mouse anti-Ncl (Santa Cruz), mouse anti-Ubf (Santa Cruz), rabbit anti-Actb (Santa Cruz), rabbit anti-Esrrb (Proteintech), rabbit anti-Klf4 (Cell Signaling), rabbit anti-Krr1 (Santa Cruz), rabbit anti-Nanog (Bethyl), rabbit anti-Pou5f1 (Abcam), rabbit anti-Tfcp2l1 (Abcam), and rabbit anti-Wdr46 (Proteintech).

Polysome profiling

For polysome fractionation, confluent R1 mESCs in 150mm dish were incubated with 100 mg/ml of cycloheximide for 10 min to arrest ribosome on mRNAs before harvesting the cells. Cells were resuspended in 1 ml of 50 mM MOPS-NaOH at pH 7.4, 150 mM NaCl, 15 mM $MgCl_2$, 0.25% Triton X-100, 1 mg/ml Heparin, 100 mg/ml cycloheximide with 5 ul protease inhibitor cocktail (Calbiochem) and 5 ul RNasin (Promega). Cells were incubated in the buffer for 10 min at 4˚C, and subjected to centrifugation for 10 min at 12,000 rpm to obtain clarified lysate. The supernatant was loaded on the 10%-50% sucrose gradient and centrifuged at 36,000 rpm for 2 hr 30 min at 4˚C with SW41T rotor (Beckman). Then, the UV

absorbance of gradient were measured at 260 nm by BioLogic LP system (BioRad).

Northern Blotting

Five ug of total RNA were resolved on agarose denaturing gels (6% formaldehyde/1.2% agarose in HEPES-EDTA buffer). Agarose gels were transferred by capillarity overnight in 10 x SSC buffer. Membrane and RNAs were irradiated by CL-1000 UV cross-linker and pre-hybridized for 30 min at 65°C in Hybrid solution containing sperm DNA. Sequence of ITS-1 probe, which is complementary to the ITS1 region of 45S pre-rRNA, is 5′-ACACACAAGACGGGGAGA-3′. The P^{32}-labeled DNA probe was added and incubated for 1 hr at 65°C and washing with first (high salt) and second (low salt) washing solution. Radioisotope signals were read with the BAS-2500 system and the signal intensities were quantified using Multi Gauge (Fujifilm).

Immunostaining

R1 cells or iPSCs were fixed in 4% formaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 2% BSA in PBS. They were incubated with the primary antibodies Nanog (Bethyl), ~~and~~ Krr1 (Santa Cruz), Sox2 (Santa Cruz), Esrrb (Proteintech), Tfcp2l1 (Abcam), and Ssea1 (Cell signaling) for 2 hr at RT. The fixed R1 cells on the slide were visualized with standard immunofluorescence technique and a fluorescence microscope (LSM700, Zeiss). The Alexa Fluor-conjugated secondary antibodies (Invitrogen) were used for visualization (1:1000). DAPI (Vector Laboratories) was used to visualize nuclei, Phalloidin (Invitrogen) was used for actin staining.

Supplemental References

Anders S, Huber W. 2010. Differential expression analysis for sequence count data. *Genome biology* **11**: R106.

Anders S, Pyl PT, Huber W. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**: 166-169.

Carey BW, Markoulaki S, Hanna J, Saha K, Gao Q, Mitalipova M, Jaenisch R. 2009. Reprogramming of murine and human somatic cells using a single polycistronic vector. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 157-162.

Cho J, Chang H, Kwon SC, Kim B, Kim Y, Choe J, Ha M, Kim YK, Kim VN. 2012. LIN28A is a suppressor of ER-associated translation in embryonic stem cells. *Cell* **151**: 765-777.

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**: 15-21.

Hansson J, Rafiee MR, Reiland S, Polo JM, Gehring J, Okawa S, Huber W, Hochedlinger K, Krijgsveld J. 2012. Highly coordinated proteome dynamics during reprogramming of somatic cells to pluripotency. *Cell Rep* **2**: 1579-1592.

Huang da W, Sherman BT, Lempicki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols* **4**: 44-57.

Ivanova N, Dobrin R, Lu R, Kotenko I, Levorse J, DeCoste C, Schafer X, Lun Y, Lemischka IR. 2006. Dissecting self-renewal in stem cells with RNA interference. *Nature* **442**: 533-538.

Lattin JE, Schroder K, Su AI, Walker JR, Zhang J, Wiltshire T, Saijo K, Glass CK, Hume DA, Kellie S et al. 2008. Expression analysis of G Protein-Coupled Receptors in mouse macrophages. *Immunome research* **4**: 5.

Liu Z, Scannell DR, Eisen MB, Tjian R. 2011. Control of embryonic stem cell lineage commitment by core promoter factor, TAF3. *Cell* **146**: 720-731.

Morgado-Palacin L, Llanos S, Serrano M. 2012. Ribosomal stress induces L11- and p53-dependent apoptosis in mouse pluripotent stem cells. *Cell cycle* **11**: 503-510.

Phipps KR, Charette J, Baserga SJ. 2011. The small subunit processome in ribosome biogenesis-progress and prospects. *Wiley interdisciplinary reviews RNA* **2**: 1-21.

Tafforeau L, Zorbas C, Langhendries JL, Mullineux ST, Stamatopoulou V, Mullier R, Wacheul L, Lafontaine DL. 2013. The complexity of human ribosome biogenesis revealed by systematic nucleolar screening of Pre-rRNA processing factors. *Molecular cell* **51**: 539-551.

Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**: 663-676.

Wang X, Seed B. 2003. A PCR primer bank for quantitative gene expression analysis. *Nucleic acids research* **31**: e154.

Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW, 3rd et al. 2009. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome biology* **10**: R130.